Nested PCR in lung tissue for diagnosis of pulmonary tuberculosis

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ABSTRACT: Diagnosis of pulmonary tuberculosis (TB) is difficult in cases with an unusual presentation and often requires a lung biopsy. The goal of this study was to determine the clinical usefulness of nested PCR on lung tissue for the diagnosis of pulmonary TB.

Clinical and laboratory data were reviewed from patients who underwent diagnostic lung biopsies, followed by nested TB PCR on formalin-fixed paraffin-embedded lung tissue specimens. The diagnostic yield and clinical impact of nested PCR were investigated.

Of the 223 patients studied, 142 were diagnosed with TB. Microbiologically confirmed TB was identified in 71 patients. Compared to culture results, the sensitivity, specificity, positive predictive value and negative predictive value of nested PCR were 85%, 99%, 98% and 88%, respectively. Nested PCR was more sensitive than acid-fast bacilli smear of respiratory specimens and histopathological findings. The PCR results provided an early diagnosis and initiation of treatment for TB. However, negative PCR results did not lead to discontinuation of unnecessary TB treatment in patients on medication.

In conclusion, nested PCR on lung tissue specimens is a useful diagnostic test for pulmonary TB in patients with an unusual presentation.

KEYWORDS: Diagnosis, lung, nested PCR, pulmonary tuberculosis, tissue

Tuberculosis (TB) is an important public health issue. Early diagnosis with subsequent treatment is necessary for successful patient outcomes. However, standard diagnostic methods, which include smear microscopy for acid-fast bacilli (AFB), have poor sensitivity and specificity, and the conventional 'gold standard' test (*i.e.* mycobacterial culture) is slow, usually requiring 2–6 weeks for results [1]. Moreover, AFB smears or mycobacterial cultures of respiratory specimens cannot be performed in patients with pulmonary TB without sputum production. In South Korea, where the prevalence of TB is intermediate, the disease remains a serious public health problem. In 2007, the number of reported TB cases was 37,554 and the estimated prevalence was 60,969 per year in South Korea [2]. However, isolation of nontuberculous mycobacteria (NTM) is rare [3]; therefore, identification of possible TB cases is more important than exclusion of NTM in South Korea.

Pulmonary TB can present with diverse clinical features, especially in Asians [4, 5]. Lung biopsy is often needed to rule out other diagnoses, and to confirm the presence of pulmonary TB, in cases with unusual radiological findings such as a solitary pulmonary nodule or a consolidation on chest computed tomography (CT).

In biopsy specimens, chronic granulomatous inflammation with central caseation is the characteristic histopathological finding of TB; however, it is not pathognomonic. Moreover, tuberculous granulomas may show atypical findings without central caseation, even in immunocompetent individuals [6]. Histological AFB staining can be used to improve the diagnostic yield in suspected cases of TB with granulomatous inflammation; however, AFB staining of histological specimens has a low sensitivity, its detection limit is $>10^4$ bacilli·slide^{-1} [7]. Furthermore, the sensitivity of AFB staining in formalin-fixed, paraffin-embedded tissues can be further reduced by the fixative fluids and organic solvents [8].

Recently, diagnostic assays, based on nucleic acid amplification methods, which include PCR, have been widely used for the rapid diagnosis of TB. The diagnostic yield and clinical utility of PCR for TB, in respiratory specimens (*i.e.* sputum or bronchial washing), is well documented [9]. However, the utility of PCR for the diagnosis of TB is uncertain in tissue specimens [1]. In tissue specimens, nested PCR, which consists of two consecutive reactions, has been used; the second reaction amplifies a DNA sequence within the first amplification product. This approach provides a higher sensitivity and specificity when compared

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with conventional single-step PCR assays [10, 11]. Currently, only one prior study has reported on the diagnostic yield of nested TB PCR, specifically in lung biopsy specimens [12]. However, there is no prior study that has evaluated the clinical significance of tissue PCR for the diagnosis of TB.

Therefore, in this study, we evaluated the clinical usefulness of nested PCR, in formalin-fixed paraffin-embedded lung biopsy specimens, for the diagnosis of pulmonary TB.

MATERIALS AND METHODS

Study design and subjects

We retrospectively reviewed the clinical and laboratory data of suspected TB patients that had a diagnostic lung biopsy, followed by nested PCR for TB on formalin-fixed paraffin-embedded lung tissue specimens. Data were collected from May 2003 to December 2008. In total, 341 patients were analysed by nested PCR of the lung biopsy specimens. To compare the diagnostic yield of the nested PCR, we included only patients who had AFB smears and mycobacterial cultures of respiratory specimens, histological AFB staining and nested PCR. Finally, 250 patients were enrolled after exclusion of 91 patients. The clinical data were reviewed for biopsy procedures, chest radiographic findings, CT findings, final diagnoses and HIV status. The typical radiological findings of TB were defined by multiple nodular or interstitial infiltrates predominantly in the upper lobes [13]. The diagnostic yield of PCR was compared to the microbiological tests and histopathological examinations. To evaluate the impact of the nested PCR on treatment decisions, the number of patients who had their management changed was analysed as well as the receipt date of the PCR results and the start date of TB medications.

At the Seoul National University Bundang Hospital (Seoul, Republic of Korea), a tertiary teaching hospital, approximately 250 new cases of TB are reported annually. During the study period, 1,434 patients were diagnosed with pulmonary TB. This study protocol was reviewed and approved by the Hospital Ethics Review Committee.

Diagnosis of pulmonary TB

The diagnostic criteria used for pulmonary TB was as follows: 1) definite TB: positive mycobacterial cultures from respiratory specimens (sputum or bronchial washing); 2) probable TB: negative mycobacterial cultures from respiratory specimens but typical histopathological findings of TB (chronic granulomatous inflammation with caseation necrosis) with good clinical response to TB medications; and 3) possible TB: none of the above, but histopathological findings suggesting TB (chronic inflammation, granulomas or necrosis alone) and good clinical response to TB medication.

AFB smear and mycobacterial culture of respiratory specimens

Sputa and bronchial aspirates (respiratory specimens) were decontaminated with 4% sodium hydroxide, homogenised and concentrated by centrifugation ($3{,}000 \times g$, 20 min). The processed sediment was stained using the Ziehl–Neelsen method. Mycobacterial cultures were established using 3% Ogawa media (Shinyang Chemical Co., Ltd, Seoul, Korea) with the concentrated specimen; they were monitored every week until 8 weeks after inoculation. The *Mycobacterium tuberculosis* complex was identified using a commercial DNA probe (AccuProbe Mycobacterium complex culture identification kit; Gen-Probe; San Diego, CA, USA).

AFB staining and nested PCR for Mycobacterium tuberculosis of lung tissue specimens

Tissue samples

The lung tissues were fixed in 4% formalin. To detect AFB, Ziehl–Neelsen staining was performed on the lung specimens in addition to haematoxylin–eosin staining; all specimens were reviewed by two pathologists. To perform the nested PCR, three 20-μm-thick sections were cut from the formalin-fixed, paraffin-embedded block. In order to prevent carry-over of contaminating DNA, a fresh blade was used for each sample and the microtome overlay was covered with a piece of adhesive tape that was changed for every sample; after processing each specimen it was cleaned with xylene and 100% ethanol. Due to the large number of samples, no more than 10 blocks were sectioned in a single batch. As a negative extraction control, three serial 20-μm-thick sections were cut from the formalin-fixed, paraffin-embedded tissue samples with a histopathological diagnosis other than mycobacterial infection; these specimens were processed in exactly the same manner as the test samples. The cut sections were collected in 1.5-mL microcentrifuge tubes and were melted at 65°C for 10 min. Paraffin was removed from the samples by adding 1 mL of xylene, vortexing the mixture and incubating the mixture at room temperature for 30 min; this was followed by 5 min of centrifugation at $9{,}015 \times g$. The supernatant was then carefully removed and discarded. An additional 1 mL of xylene was added to the pellet, and the procedure was repeated. To facilitate pelletting and hydration of the samples, 1 mL of 100% ethanol was added. After vortexing, the samples were pelleted by centrifugation at $9{,}015 \times g$ for 5 min and the supernatant was removed. The pellet was then air dried.

DNA extraction

DNA extraction and nested PCR for *M. tuberculosis* was performed using the commercially available ABSOLUTE MTB PCR kits (BioSewoom Inc., Seoul, Korea) [14–16], according to the manufacturer's instructions. The samples were resuspended in 300 μL of digestion buffer made up of 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 0.5% sodium dodecyl sulfate, 50 mM NaCl, and 300 $\mu g \cdot mL^{-1}$ of proteinase K, and the mixture was incubated at 37°C under rocking conditions for at least 24–48 h until most of the tissue was disintegrated [17, 18].

The proteinase K was inactivated by incubating the samples at 95°C for 10 min. DNA was extracted from the emulsified tissue samples by adding 300 μL of phenol, vortexing the mixture, and centrifuging the mixture at $10{,}581 \times g$ for 3 min. The supernatant was then removed and transferred into a new tube to which 300 μL of phenol–chloroform (1:1) was added. After vortexing and centrifuging once again at $10{,}581 \times g$ for 3 min, the supernatant was transferred into a new vial and 300 μL of chloroform-isoamyl alcohol (24:1) was added. After transferring the supernatant to a new tube, sodium acetate at a final concentration of 0.2 M and 99% ice-cold ethanol (500 mL) were added to precipitate the nucleic acids.

Nested PCR

The PCR procedure used was a nested PCR based on the amplification of the repeated insertion sequence IS*6110*. The first round using outer primers amplified a 256-bp fragment and the second-round PCR using the inner primers amplified a 181-bp fragment. The total reaction volume in the first PCR round was 15.5 μL, and the reaction mixture contained each dNTP at a concentration of 250 mM, 1 mM MgCl2, 1.25 U of *Taq* DNA polymerase, 0.5 μLof *Taq* polymerase buffer (supplied with the enzyme) and primers at 0.3 mM each. The total reaction volume in the second round was 18.5 μL and contained *Taq* polymerase buffer (supplied with the enzyme), each dNTP at a concentration of 125 mM, 1.5 mM $MgCl_2$, 1.25 U of *Taq* polymerase and primers at 0.3 mM each. DNA sample (4.5 μL) was added to the reaction mixture in the first round. Both PCR rounds were conducted with an initial 4 min denaturation step at 94°C coupled to a repeating cycle of 30 s at 94°C, 30 s at 68°C, and 30 s at 72°C for 35 (first round) and 25 (second round) cycles, followed by 5 min of final extension at 72°C. A total of 1.5 μL of the first-round PCR product was transferred to the second-round PCR mixture.

Detection of PCR products

For analysis of the amplified products of each PCR assay performed, 10 μL of the reaction solutions from the second round of amplification were resolved on 2% agarose gels containing 1 $mg \cdot mL^{-1}$ of ethidium bromide, and the products were visualised by UV transillumination.

Statistical methods

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio of a positive test result (LR+) and likelihood ratio of a negative test result (LR-) for the diagnosis of pulmonary TB were calculated for AFB smears of respiratory specimens, histological AFB staining, pathological criteria alone, and nested PCR results. 95% confidence intervals (CIs) were estimated according to the binomial distribution. A Chi-squared test was used to compare the positive proportions and significance was set at $p<0.05$. Concordance between test results was assessed using κ coefficients ($\kappa>0.75$, excellent agreement; $0.75 \geqslant \kappa \geqslant 0.40$, fair to good agreement; $\kappa<0.40$, poor agreement). Analyses were performed using SPSS 12.0 statistical software (SPSS; Chicago, IL, USA) and the internet statistical program 2-Way Contingency Table Analysis (http://statpages.org/ctab2x2.html).

RESULTS

Baseline characteristics of the study patients

In total, 250 patients were enrolled. 27 patients who had negative mycobacterial culture results and were lost to follow-up were excluded, as their final diagnoses were unknown. After exclusions, 223 patients were included in the analysis. Most of the study patients were referred from primary care clinics due to abnormal radiological findings with an uncertain diagnosis. The included patients belonged to one of the following criteria: patients with initial negative AFB smear results on sputum despite typical radiological findings of TB or patients who showed clinical symptoms compatible with TB but had unusual radiological findings for TB. Therefore, they required a lung biopsy to confirm the diagnosis of TB and to rule out other possible diagnoses. The baseline characteristics of the study patients are shown in table 1. Of the 35 patients that had a surgical lung biopsy, 13 underwent transthoracic needle biopsy (TTNB) or transbronchial lung biopsy (TBLB) before the surgery. However, the results of the nonsurgical biopsy specimens were not informative due to insufficient tissue samples. Therefore, additional surgical lung biopsies were necessary.

Diagnostic performance of the nested PCR on lung biopsy specimens

The diagnostic performance of the nested PCR was analysed according to the diagnostic standards used for TB (table 2). The overall sensitivity, specificity, PPV and NPV of the nested PCR were 85%, 99%, 98% and 88%, respectively, compared with the mycobacterial cultures used as the gold standard. Including all cases of probable and possible TB, the sensitivity and NPV were decreased to 66% and 63%. Nested PCR had 11 cases with false negative results compared with mycobacterial culture. All 11 patients underwent non-surgical biopsies (10 with TTNB

TABLE 1 Baseline characteristics of the study patients

Patients n	223
Sex	
Male	149 (66.8)
Female	74 (33.2)
Age yrs	57 (19–89)
Biopsy procedures	
Transthoracic needle biopsy	170 (76.2)
Surgical biopsy	35 (15.7)
Transbronchial lung biopsy	18 (8.1)
Chest radiography findings[#]	
Typical	30 (13.5)
Atypical	193 (86.5)
CT findings	
Nodule	73 (32.7)
Mass	31 (13.9)
Consolidation	54 (23.3)
Cavity	52 (23.3)
Other[¶]	13 (5.8)
Final diagnosis	
TB	142 (63.7)
Definite TB	71 (31.8)
Probable TB	24 (10.8)
Possible TB	47 (21.1)
Non-specific inflammatory lesion	32 (14.3)
Lung cancer	13 (5.8)
Other[+]	36 (16.1)
HIV-positive[§]	0

Data are presented as n (%) or median (range), unless otherwise stated. CT: computed tomography; TB: tuberculosis. [#]: typical chest radiographic findings were defined as multiple nodular or interstitial infiltrates predominantly in the upper lobes; [¶]: CT findings of miliary or disseminated nodules (n=6), diffuse ground-glass opacities (n=6), and bulla (n=1); [+]: final diagnosis of sarcoidosis (n=9), nontuberculous mycobacterial infection (n=13), cryptogenic organising pneumonia (COP) (n=3), fungal infection (n=3), paragonimiasis (n=2), interstitial lung disease other than COP (n=4), actinomycosis (n=1), and lymphoma (n=1); [§]: HIV tests were carried out in 204 (91.5%) patients and none of them showed positive results.

and one with TBLB) and only one out of the 11 had typical histopathological findings of TB (chronic granulomatous inflammation with caseation necrosis). There was one case of a false-positive nested PCR result, a lung abscess. Despite the positive PCR, medication for TB was not used in this patient with a lung abscess because the clinical course was more compatible with a bacterial infection than with TB.

The diagnostic performance of the nested PCR was evaluated in association with the histopathological findings and biopsy procedures, using mycobacterial cultures as the gold standard (table 3). The sensitivity was more increased in cases with typical histopathological findings, positive histological AFB staining and surgical biopsy specimens compared with cases with atypical histopathological findings, negative histological AFB staining and nonsurgical biopsy specimens.

Comparison of nested PCR results with AFB smears of respiratory specimens and histopathological findings

The diagnostic yield of the nested PCR was compared with the AFB smears of the respiratory specimens and with the histopathological findings (table 4). The diagnostic yields were calculated using mycobacterial cultures as the gold standard.

The sensitivity of the nested PCR (85%) was higher than the other methods (27–39%). The LR+ of the nested PCR (68.5, 95% CI 14.5–386.9) and the LR- of the nested PCR (0.16, 95% CI 0.14–0.22) were superior compared with the other tests. Next, the concordance between the nested PCR and the other tests was calculated (table 5). The nested PCR for TB showed fair to good agreement with the mycobacterial cultures ($\kappa=0.564$, $p<0.001$) and the histological AFB staining ($\kappa=0.542$, $p<0.001$).

Clinical impact of the lung tissue nested PCR for the diagnosis and treatment of pulmonary TB

To evaluate the clinical impact of the nested PCR, we documented the changes in treatment decisions after receipt of the PCR results (table 6).

In 60 patients with definite TB, 52 patients continued TB medication with confidence, after confirmation by the positive PCR results. Furthermore, a positive PCR result allowed eight patients who initially had a low probability of TB to start proper treatment. There were 11 patients with TB who had a negative PCR result. Ten of the 11 patients had received TB medication before the PCR results were reported. After the PCR was reported as negative, the physicians continued treatment in these patients. Among the patients who had been started on TB medication prior to receipt of the PCR results, 10 patients were ultimately diagnosed as ''not having TB''. After the PCR results were reported as negative, only one patient discontinued the TB medication; nine patients continued treatment until the culture revealed NTM. In addition, treatment was started in two patients despite a negative PCR; they were eventually confirmed as NTM. The positive PCR results allowed patients on treatment to continue medication without doubt and the untreated patients to start treatment. However, negative PCR results did not lead to decisions to discontinue medication in patients already on treatment.

In addition, we analysed the time from the initial evaluation to the report of the mycobacterial cultures and to the PCR results. The mean time from the first evaluation to the report of results was 17.1 ± 13.6 days for PCR and 54.9 ± 22.1 days for the mycobacterial culture. The nested PCR provided a diagnosis earlier than the mycobacterial cultures.

For the purpose of determining the cost of the nested TB PCR, the annual costs associated with the nested PCR testing at our pathology laboratory were estimated. The costs were calculated at the exchange rate of 1,200 South Korean won to US $1.00. The nested TB PCR kits are sold with a set of 25 tests at a cost of $302.5 per set and about 50 tests are performed per month. The cost of supplies (such as gloves and microcentrifuge tubes) and the reagents used for PCR, were estimated at $500 per year. The nested PCR is performed once a week at our centre. We calculated that a nested PCR test requires 3 h of

TABLE 2 Diagnostic performance of nested PCR on lung tissue specimens by the diagnostic standards used for pulmonary tuberculosis (TB)

Diagnostic standards	TB	Not TB	Total	Sensitivity	Specificity	PPV	NPV
Mycobacterial culture[#]				85 (80–86)	99 (95–100)	98 (93–100)	88 (84–89)
PCR positive	60	1[§]	61				
PCR negative	11	80	91				
Total	71	81	152				
Mycobacterial culture or histopathology[¶]				78 (74–79)	99 (94–100)	99 (94–100)	79(76–80)
PCR positive	74	1[§]	75				
PCR negative	21	80	101				
Total	95	81	176				
Mycobacterial culture, histopathology or medication response[+]				66 (63–67)	99 (94–100)	99 (95–100)	63 (59–63)
PCR positive	94	1[§]	95				
PCR negative	48	80	128				
Total	142	81	223				

Data are presented as n or % (95% CI). PPV: positive predictive value; NPV: negative predictive value. [#]: culture-positive: definite TB; [¶]: typical histopathological findings for TB with good response to TB medication: probable TB; [+]: good response to TB medication with culture negative and atypical histopathological findings: possible TB; [§]: one case of a lung abscess.

TABLE 3 Diagnostic performance of nested PCR on lung tissue specimens according to the histopathological findings and biopsy procedures

	TB		Not TB		Sensitivity	Specificity	PPV	NPV
	PCR+	PCR-	PCR+	PCR-				
Specimens n	71		81					
Histopathological findings								
Typical[#]	18	1	0	2	95 (88–95)	100 (39–100)	100 (93–100)	67 (26–67)
Atypical[¶]	42	10	1[+]	78	81 (74–82)	99 (95–100)	98 (91–100)	89 (85–90)
Histological AFB staining								
Positive	39	2	0	5	95 (91–95)	100 (64–100)	100 (95–100)	71 (45–71)
Negative	21	9	1[+]	75	70 (60–73)	99 (95–100)	96 (81–99)	89 (86–90)
Biopsy procedure								
Surgical biopsy	3	0	0	16	100 (56–100)	100 (92–100)	100 (56–100)	100 (92–100)
Transthoracic needle biopsy	53	10	1[+]	55	84 (79–85)	98 (92–100)	98 (92–100)	85 (80–86)
Transbronchial lung biopsy	4	1	0	9	80 (49–80)	100 (83–100)	100 (61–100)	90 (74–90)

Data are presented as n or % (95% CI). TB: tuberculosis; PPV: positive predictive value; NPV: negative predictive value; AFB: acid-fast bacilli. [#]: chronic granulomatous inflammation with caseation necrosis; [¶]: histopathological findings that were not typical for the pathology of TB but with findings suggestive of TB such as chronic inflammation, a granuloma or necrosis alone; [+]: one case with a lung abscess.

technician time at a wage of $15.60 per hour for medical technologists at our hospital. The cost per test was obtained by dividing the annual cost by the number of nested TB PCRs per year. Finally, the nested TB PCR is expected to cost approximately $16.60.

DISCUSSION

Many prior studies have reported on the performance of PCR on various types of formalin-fixed, paraffin-embedded human tissues [11, 19–21]. In these reports, the sensitivity of the PCR ranged 66–100% and varied according to the method of PCR, target sequence and characteristics of the tissue specimens. Only one prior study has reported on PCR for TB, specifically in formalin-fixed paraffin-embedded lung tissue specimens, and it reported a sensitivity of 64% [12]. However, the study had a small sample size (25 specimens) and focused mostly on the laboratory diagnostic yield of the PCR and the detection rate of *M. tuberculosis* DNA in the tissue specimens.

In our study, we evaluated the diagnostic yield of nested PCR in a large number of pure lung tissue specimens. In comparison to microbiological cultures, the sensitivity, specificity, PPV and NPV of the nested PCR for TB were 85%, 99%, 98% and 88%, respectively; comparable to other studies. Moreover, the nested PCR had a higher sensitivity and likelihood ratio of a positive test than the AFB smears of respiratory specimens, histological AFB staining and pathological criteria alone.

Nested PCR for TB was more sensitive in the lung tissue specimens with typical histopathological findings of TB and positive AFB staining. In respiratory specimens, it is well known that the sensitivity of PCR is greater in AFB smear-positive samples [1, 22]. The tissue nested PCR was more sensitive in AFB positively stained tissue, similar to the PCR in respiratory samples. In addition, the sensitivity of the PCR increased with surgical biopsy specimens compared to the nonsurgical biopsy specimens. These findings are consistent with those of a previous study reported by PEROSIO and FRANK [12], where mycobacterial DNA was detected by nested PCR for TB in seven out of seven wedge resections and nine out of 18 TBLB specimens from patients with suspected pulmonary TB. It is thought that a surgical biopsy specimen allows for a better sample with a sufficient amount of tissue containing mycobacterial DNA, and thus increases the sensitivity of the PCR. In our

TABLE 4 Diagnostic yields of acid-fast bacilli (AFB) smears of respiratory specimens, histological AFB staining, pathological criteria alone and nested PCR

	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Sputum/bronchial washing AFB smear	39 (33–43)	94 (88–97)	85 (71–93)	64 (60–66)	6.4 (2.8–15.5)	0.65 (0.58–0.76)
Histological AFB staining	58 (51–62)	94 (88–97)	89 (79–95)	72 (67–74)	9.4 (4.3–22.0)	0.45 (0.40–0.56)
Pathological criteria[#]	27 (21–29)	98 (93–99)	91 (73–97)	60 (57–61)	10.8 (3.0–41.5)	0.75 (0.72–0.85)
Nested PCR	85 (80–86)	99 (95–100)	98 (93–100)	88 (84–89)	68.5 (14.5–386.9)	0.16 (0.14–0.22)

Data are presented as % (95% CI). Diagnostic yields were calculated using mycobacterial cultures as the gold standard. PPV: positive predictive value; NPV: negative predictive value; LR+: likelihood ratio of a positive test; LR-: likelihood ratio of a negative test. [#]: typical pathology of tuberculosis chronic granulomatous inflammation with caseation necrosis.

TABLE 5 Concordance of nested PCR with acid-fast bacilli (AFB) smear/culture of respiratory specimens and histopathological findings in all study patients

	Nested PCR		κ	p-value
	Positive	Negative		
Patients n	223	223		
Sputum/bronchial washing AFB smear			0.264	<0.001
Positive	29	8		
Negative	66	120		
Sputum/bronchial washing mycobacterial culture			0.564	<0.001
Positive	60	11		
Negative	35	117		
Histological AFB stain			0.542	<0.001
Positive	57	10		
Negative	38	118		
Pathological criteria[#]			0.101	0.045
Positive	13	6		
Negative	67	85		

Data are presented as n, unless otherwise stated. [#]: typical pathology of tuberculosis chronic granulomatous inflammation with caseation necrosis.

study, there were 11 culture-positive TB patients with negative PCR results. All of them underwent a nonsurgical lung biopsy. The false-negative PCR may have resulted from an inadequate amount of tissue obtained by the nonsurgical biopsy. Therefore, it may be important to obtain appropriate and sufficient amounts of tissue specimen to increase the diagnostic sensitivity of the nested TB PCR.

The clinical impact and the cost of nested PCR were investigated in addition to the laboratory diagnostic yield. Nested PCR provided a diagnosis about 37 days earlier than the mycobacterial culture. The time from the initial evaluation to the receipt of the nested PCR results included the time for admission, for performing the biopsy and for the availability of PCR analysis (PCR was performed once a week). Despite such factors, PCR provided a more rapid diagnosis of TB than culture. Moreover, the cost of the tissue nested PCR was reasonable. However, more rapid and sensitive culture media and automated culture systems have recently been developed [23]. In our hospital, liquid media was newly introduced and a simultaneous liquid and Ogawa media culture system has been used since September 2008. Further studies, comparing PCR with the new liquid media culture system, are needed to clearly demonstrate the diagnostic utility of PCR.

In this study, the physicians tended to start empirical treatment for TB in patients with suspicious granulomatous inflammation regardless of the PCR results. A total of 72 patients were started on TB medication immediately after receipt of the pathology report, before the PCR was reported. Nine patients continued treatment until the culture revealed NTM despite a negative PCR. Even though the tissue nested PCR revealed excellent specificity and greater sensitivity than AFB staining and histopathology criteria, it showed suboptimal sensitivity and NPV compared with the culture results. Because of the insufficient sensitivity and NPV of the PCR, empirical TB treatment might be inevitable in patients with suspected TB despite a negative PCR, especially in South Korea where the prevalence of TB is intermediate.

There were 34 probable or possible TB patients in whom the PCR was positive, despite a negative culture. Several studies have reported the possibility of no cultural growth secondary

TABLE 6 Treatment decisions for definite tuberculosis (TB) patients and patients without TB according to the nested PCR results

	Treated prior to receipt of PCR result		Treated after receipt of PCR result		Total
	Continued	Stopped	Started	Not started	
Definite TB					
PCR positive	52	0	8	0	60
PCR negative	10	0	0	1	11
Not TB					
PCR positive	0	0	0	1[#]	1
PCR negative	9[¶]	1[+]	2[¶]	68	80

[#]: lung abscess; [¶]: nontuberculous mycobacteria; [+]: sarcoidosis.

to specific therapy and the persistence of PCR positivity several weeks after the initiation of treatment [24, 25]. In our study, 11 of the 34 patients had a previous history of TB. However, all of the patients had respiratory symptoms or an expanding lesion on chest radiography, which suggests active TB. All of the 34 patients were started on TB treatment and responded well to the treatment.

This study had several limitations. First, it was a retrospective study; therefore, the diagnostic work-up did not have an identical protocol and the indications for the lung biopsy were